Amdt. dated December 17, 2008

Reply to Office Action of February 20, 2008

## Amendments to the Claims:

## 1. (canceled)

- 2. (currently amended) A method of assaying a *Brassica* plant for imidazolinone herbicide tolerance conferred by the PM1 mutation of the *B. napus AHAS1* gene, the method comprising the steps of:
  - a) isolating genomic DNA from the plant;
  - b) selectively amplifying an *AHAS1* gene from the genomic DNA using an *AHAS1* forward primer having a the sequence as set forth in nucleotides 1 to 22 of SEQ ID NO:9 and an *AHAS1* reverse primer in a first amplification step, thereby producing an *AHAS1* reaction mixture;
  - c) removing the *AHAS1* primers from the *AHAS1* reaction mixture to produce a purified *AHAS1* reaction mixture;
  - d) in a second amplification step, further amplifying a portion of the amplified *AHAS1* gene containing the site of the PM1 mutation, by combining the purified *AHASI* reaction mixture with a PM1 forward primer and a PM1 reverse primer, wherein the PM1 forward primer and the PM1 reverse primer bind to sites nested within the amplified portion of the *AHAS1* gene;
  - e) denaturing the product of the second amplification step to produce single stranded polynucleotides that are allowed to adopt unique conformations by intramolecular interactions; and
  - f) detecting the presence or absence of the PM1 mutation on the basis of the mobility of said single stranded polynucleotide conformers in a substrate.

Amdt. dated December 17, 2008

- 3. (currently amended) A method of assaying a *Brassica* plant for imidazolinone herbicide tolerance conferred by the PM1 mutation of the *B. napus AHAS1* gene, the method comprising the steps of:
  - a) isolating genomic DNA from the plant;
  - b) selectively amplifying an *AHAS1* gene from the genomic DNA using an *AHAS1* forward primer and an *AHAS1* reverse primer having a the sequence as set forth in nucleotides 1 to 22 of SEQ ID NO:10 in a first amplification step, thereby producing an *AHAS1* reaction mixture;
  - c) removing the *AHAS1* primers from the *AHAS1* reaction mixture to produce a purified *AHAS1* reaction mixture;
  - d) in a second amplification step, further amplifying a portion of the amplified *AHAS1* gene containing the site of the PM1 mutation, by combining the purified *AHAS1* reaction mixture with a PM1 forward primer and a PM1 reverse primer, wherein the PM1 forward primer and the PM1 reverse primer bind to sites nested within the amplified portion of the *AHAS1* gene;
  - e) denaturing the product of the second amplification step to produce single stranded polynucleotides that are allowed to adopt unique conformations by intramolecular interactions; and
  - f) detecting the presence or absence of the PM1 mutation on the basis of the mobility of said single stranded polynucleotide conformers in a substrate.
- 4. (previously presented) The method of claim 2 or 3, wherein the PM1 forward primer has a sequence as set forth in nucleotides 1 to 21 of SEQ ID NO:11.
- 5. (previously presented) The method of claim 2 or 3, wherein the PM1 reverse primer has a sequence as set forth in nucleotides 1 to 21 of SEQ ID NO:12.

Amdt. dated December 17, 2008

Reply to Office Action of February 20, 2008

6. (previously presented) The method of claim 2 or 3, wherein step (d) includes incorporating a label into the amplified portion of the *AHAS1* gene.

7. (original) The method of claim 6, wherein the label is selected from the group consisting of a radioactive label, a fluorescent label, a luminescent label, and a paramagmetic label.

8. (previously presented) The method of claim 2 or 3, wherein the substrate is selected from the group consisting of polyacrylamide, linear polyacrylamide, poly(N,N-dimethylacrylamide), hydroxyalkyl cellulose, polyoxyethylene, F127, agarose, diethylaminoethyl cellulose, sepharose, POP4, and POP6.

9. (previously presented) The method of claim 2 or 3, wherein the detection method is selected from the group consisting of electrophoresis and chromatography.

10. (previously presented) The method of claim 2 or 3, further comprising the step of detecting the presence or absence of PM2-mediated imidazolinone resistance in the plant.

## 11. (canceled)

- 12. (currently amended) A method for assaying a *Brassica* plant for imidazolinone herbicide tolerance conferred by the PM2 mutation of the *B. napus AHAS3* gene, the method comprising the steps of:
  - a) isolating genomic DNA from the plant;
  - b) selectively amplifying the *AHAS3* gene from the genomic DNA using an *AHAS3* forward primer having a <u>the</u> sequence as set forth in nucleotides 1 to 22 of SEQ ID NO:13 and an *AHAS3* reverse primer in a first amplification step to produce an *AHAS3* reaction mixture;
  - c) removing the *AHAS3* primers from the *AHAS3* reaction mixture to produce a purified *AHAS3* reaction mixture;

Amdt. dated December 17, 2008

- d) in a second amplification step, further amplifying the amplified *AHAS3* gene, by combining a first aliquot of the purified *AHAS3* reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for a wild type allele of the PM2 region at position 1712 of the *AHAS3* gene as depicted in SEQ ID NOs:5 and 8;
- e) in a third amplification step further amplifying the amplified AHAS3 gene, by combining a second aliquot of the purified AHAS3 reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for the PM2 mutation; and
- f) analyzing the amplified first and second aliquots for the presence or absence of the PM2 mutation.
- 13. (currently amended) A method for assaying a *Brassica* plant for imidazolinone herbicide tolerance conferred by the PM2 mutation of the *B. napus AHAS3* gene, the method comprising the steps of:
  - a) isolating genomic DNA from the plant;
  - b) selectively amplifying the *AHAS3* gene from the genomic DNA using an *AHAS3* forward primer and an *AHAS3* reverse primer having a the sequence as set forth in nucleotides 1 to 23 of SEQ ID NO:14 in a first amplification step to produce an *AHAS3* reaction mixture;
  - c) removing the *AHAS3* primers from the *AHAS3* reaction mixture to produce a purified *AHAS3* reaction mixture;
  - d) in a second amplification step, further amplifying the amplified *AHAS3* gene, by combining a first aliquot of the purified *AHAS3* reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for a wild type allele of the PM2 region at position 1712 of the *AHAS3* gene as depicted in SEQ ID NOs:5 and 8;
  - e) in a third amplification step further amplifying the amplified *AHAS3* gene, by combining a second aliquot of the purified *AHAS3* reaction mixture with a

Amdt. dated December 17, 2008

Reply to Office Action of February 20, 2008

PM2 region forward primer, a PM2 region reverse primer, and a primer selective for the PM2 mutation; and

- f) analyzing the amplified first and second aliquots for the presence or absence of the PM2 mutation.
- 14. (previously presented) The method of claim 12 or 13, wherein the PM2 region forward primer has a sequence as set forth in nucleotides 1 to 19 of SEQ ID NO:15.
- 15. (previously presented) The method of claim 12 or 13, wherein the PM2 region reverse primer has a sequence as set forth in nucleotides 1 to 19 of SEQ ID NO:16.
- 16. (previously presented) The method of claim 12 or 13, wherein the wild type allele of the PM2 region at position 1712 has a sequence as set forth in nucleotides 1 to 18 of SEQ ID NO:17.
- 17. (previously presented) The method of claim 12 or 13, wherein the primer selective for the PM2 mutation has a sequence as set forth in nucleotides 1 to 20 of SEQ ID NO:18.
- 18. (previously presented) The method of claim 12 or 13, wherein steps (d) and (e) include incorporating a label into the amplified portion of the *AHAS3* gene.
- 19. (original) The method of claim 18, wherein the label is selected from the group consisting of a radioactive label, a fluorescent label, a luminescent label, and a paramagmetic label.
- 20. (previously presented) The method of claim 12 or 13, wherein the analyzing step employs a method selected from the group consisting of electrophoresis and chromatography.

Amdt. dated December 17, 2008

- 21. (previously presented) The method of claim 12 or 13, further comprising the steps of:
  - g) selectively amplifying an *AHAS1* gene from the genomic DNA using an *AHAS1* forward primer and an *AHAS1* reverse primer in a fourth amplification step;
  - h) removing the AHAS1 primers from the product of step g);
  - i) in a fifth amplification step, further amplifying a portion of the amplified *AHAS1* gene containing the site of the PM1 mutation, by combining the product of step h) with a PM1 forward primer and a PM1 reverse primer, wherein the PM1 forward primer and the PM1 reverse primer bind to sites nested within the amplified portion of the *AHAS1* gene;
  - j) denaturing the product of the fifth amplification step to produce single stranded polynucleotides that are allowed to adopt unique conformations by intramolecular interactions; and
  - k) detecting the presence or absence of the PM1 mutation on the basis of the mobility of said single stranded conformer polynucleotides in a substrate.
- 22. (canceled)
- 23. (canceled)
- 24. (currently amended) A method of marker assisted breeding of plants of *Brassica* species using a PM1 mutation of the *B. napus AHAS1* gene as a marker, the method comprising the steps of:
  - a) isolating genomic DNA from a *Brassica* plant;
  - b) selectively amplifying an *AHAS1* gene from the genomic DNA using an *AHAS1* forward primer having a <u>the</u> sequence as set forth in nucleotides 1 to 22 of SEQ ID NO:9 and an *AHAS1* reverse primer in a first amplification step, thereby producing an *AHAS1* reaction mixture;

Amdt. dated December 17, 2008

- c) removing the *AHAS1* primers from the *AHAS1* reaction mixture to produce a purified *AHAS1* reaction mixture;
- d) in a second amplification step, further amplifying a portion of the amplified *AHAS1* gene containing the site of the PM1 mutation, by combining the purified *AHAS1* reaction mixture with a PM1 forward primer and a PM1 reverse primer, wherein the PM1 forward primer and the PM1 reverse primer bind to sites nested within the amplified portion of the *AHAS1* gene;
- e) denaturing the product of the second amplification step to produce single stranded polynucleotides that are allowed to adopt unique conformations by intramolecular interactions;
- f) detecting the presence or absence of the PM1 mutation on the basis of the mobility of said single stranded polynucleotide conformers in a substrate; and
- g) selecting said plant as a parent for further breeding if the PM1 mutation is present.
- 25. (currently amended) A method of marker assisted breeding of plants of *Brassica* species using a PM2 mutation of the *B. napus AHAS3* gene as a marker, the method comprising the steps of:
  - a) isolating genomic DNA from the plant;
  - b) selectively amplifying the *AHAS3* gene from the genomic DNA using an *AHAS3* forward primer and an *AHAS3* reverse primer having a the sequence as set forth in nucleotides 1 to 22 23 of SEQ ID NO:10 14 in a first amplification step to produce an *AHAS3* reaction mixture;
  - c) removing the *AHAS3* primers from the *AHAS3* reaction mixture to produce a purified *AHAS3* reaction mixture;
  - d) in a second amplification step, further amplifying the amplified *AHAS3* gene, by combining a first aliquot of the purified *AHAS3* reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for a wild type allele of the PM2 region at position 1712 of the *AHAS3* gene as depicted in SEQ ID NOs:5 and 8;

Amdt. dated December 17, 2008

Reply to Office Action of February 20, 2008

e) in a third amplification step further amplifying the amplified *AHAS3* gene, by combining a second aliquot of the purified *AHAS3* reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for the PM2 mutation;

- f) analyzing the amplified first and second aliquots for the presence or absence of the PM2 mutation; and
- f) selecting said plant as a parent for further breeding if the PM2 mutation is present.